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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C07K 13/00, A61K 39/00, 39/395
A61K 9/127, 47/48

A1

(11) International Publication Number: WO 94/10198

(43) International Publication Date: 11 May 1994 (11.05.94)

(21) International Application Number: PCT/EP93/03073

(22) International Filing Date: 2 November 1993 (02.11.93)

(30) Priority data:
970,416 2 November 1992 (02.11.92) US

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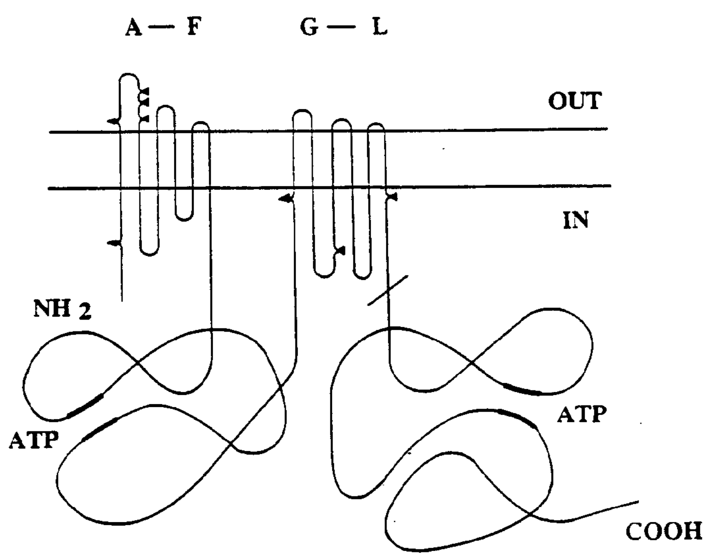
(81) Designated States: AU, BB, BG, BR, BY, CA, CZ, FI, HU, JP, KP, KR, KZ, LK, MG, MN, MW, NO, NZ, PL, RO, RU, SD, SK, UA, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD OF REDUCING MULTIDRUG RESISTANCE IN CELLS AND TISSUES



(57) Abstract

Methods and vaccine compositions for reducing or inhibiting multidrug resistance in cancer cells and tissues, and more particularly antigenic P-glycoprotein-containing compositions that, when administered to a human or animal with a multidrug resistance cancer or tumor, elicit the production of antibodies specific for P-glycoprotein. These antibodies reduce multidrug resistance, thus making the cancer or tumor more sensitive to anti-cancer drugs. The present invention encompasses P-glycoprotein-containing compositions including liposomes having portions of P-glycoprotein externally presented on their surfaces, P-protein peptide fragments, modified P-protein fragments and liposomes containing P-protein peptide fragments and/or modified P-protein peptide fragments. The present invention also includes antibodies specific for externally accessible regions or epitopes of the P-protein. Thus, according to the present invention, multidrug resistance in cells and tissues can be reduced either by active immunization of an individual having multidrug resistance using antigenic P-glycoprotein-containing compositions or by passive immunization via administering an antibody or a group of antibodies specific for P-protein epitopes.

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"METHOD OF REDUCING MULTIDRUG RESISTANCE IN CELLS AND TISSUES"

Technical Field

The present invention relates to vaccines for reducing multidrug resistance in cells and tissues, especially cancer cells. More particularly, the present invention relates to a method of vaccinating against certain portions of P-protein and using the vaccines to to reduce multidrug resistance in cancer cells *in vivo*.

Background of the Invention

While many cancers have been treated successfully by chemotherapeutic agents, a significant number of cancers are intrinsically drug resistant and others acquire resistance following chemotherapy. Cancers are frequently resistant to more than one type of drug. This phenomenon is called multidrug resistance or MDR. The recognition that a complex drug resistance phenotype of broad specificity in human tumors could limit seriously successful chemotherapy has provided the incentive to study MDR cell lines as a model for clinical drug resistance.

The most common form of MDR is resistance to many natural products drugs with widely different structures and different mechanisms of action. In Table. 1. Pastan et al show a few drugs used in the treatment of cancer in relation with the

multidrug resistance to them. (See Pastan, I., Willingham, M.C., & Gottesman, M. (1991) *FASEB* J. 5, 2523-2528.)

Table I

Natural Product Drugs Used to Treat Cancer

Drug	Intracellular Target	MDR
Doxorubicin	DNA; topoisomerase II	Yes
Daunomycin	DNA; topoisomerase II	Yes
Camptotechin	Topoisomerase I	No
Vinblastine	Tubulin	Yes
Vincristine	Tubulin	Yes
Taxol	Tubulin	Yes
Actinomycin D	RNA transcription	Yes
Bleomycin	DNA damage	No

The study of MDR *in vivo* has been more difficult to quantify; nonetheless, highly drug resistant cells to one or several of the compounds listed in Table I have been obtained and extensive studies have been conducted on those cells. Several alternative explanations of the reduction of intracellular drug concentration have been proposed, but two hypotheses have received most of the attention of the scientific community. It has been speculated that P-protein may be directly involved in drug transport in multidrug-resistant cells, either as an efflux pump or by changing permeability of the lipid bilayer. For a general review of P-protein, see Endicott, J.A., et al. "The Biochemistry of P-Glycoprotein-Mediated Multidrug Resistance", *Ann. Rev. Biochem.* (1989), 58:127-71.

It has emerged that a net decrease of the intracellular concentrations of drugs underlies the multiple drug-resistant phenotype. Analysis of different multidrug-resistant cell lines has indicated that the phenomenon of multidrug resistance is due to decreased drug accumulation in the resistant cells. Studies of drug transport in different multidrug-resistant cell lines have suggested that decreased drug accumulation in the resistant cells results in part from an increased rate of drug efflux occurring by an energy-dependent mechanism. Further, drug accumulation is

enhanced by metabolic inhibitors. Studies have led to the concept of an energy-dependent efflux pump responsible for the removal of various lipophilic compounds from multidrug resistant cells.

Irrespective of the mechanism causing MDR in cells, the most common biochemical characteristic of multidrug-resistant cells is the increased expression of a membrane glycoprotein with a molecular weight of approximately 170,000 Daltons. The size of P-protein, sometimes referred to as P-glycoprotein, in the absence of N-glycosylation has been estimated as approximately 140,000 Daltons. Using cDNA clones for the hamster P-protein, several investigators have shown that the P-protein gene is amplified in multidrug-resistant cell lines, and that gene amplification is accompanied by increased expression of 4.5 to 5.0 kb P-protein mRNA. Differential amplification of DNA sequences hybridizing to P-protein clones has suggested that P-proteins may be encoded by a multigene family.

Studies on expression of MDR1 mRNA in normal tissues indicate substantial expression in colon, small intestine, kidney, liver, and adrenal glands with very low level expression in most other tissues. P-protein is located on the biliary surface of hepatocytes and small biliary ductules, on the brush border of renal proximal tubule cells, on the lumenal surface of pancreatic ductules, on the lumenal surface of the mucosa of the small and large intestine, and diffusely in both medulla and cortex of the adrenal. MDR RNA is found in a similar location in the gastrointestinal tract in the hamster by *in situ* hybridization. In the mouse, an MDR1-related gene is also expressed at high levels in secretory epithelial cells in the gravid uterus. This localization is consistent with a role for P-protein as a transporter in most of these organs. Liver, kidney, and bowel are the sites from which cytotoxic natural products present in the diet, or introduced by the chemotherapist, are removed from the body. Hence, the MDR1 gene product could be functioning as a multidrug transporter in these locations to protect animals from a variety of

cytotoxic compounds present in their diets. In rat liver, levels of MDRl RNA increase after hepatectomy or after treatment with chemical carcinogens, supporting the idea that expression of the MDR1 gene is a response to toxic insult. In the adrenal gland or the pregnant uterus, the MDRl gene product may also be involved in transport of endogenous metabolites.

Adenocarcinomas derived from adrenal, kidney, liver, and bowel are known to be intrinsically resistant to a broad range of chemotherapeutic agents, including the drugs to which multidrug-resistant cells are resistant. These tumors express easily detectable levels of MDRl mRNA, suggesting that their multidrug-resistant phenotype is related to the presence of the multidrug transporter. For kidney tumors, this concept is supported by evidence that unselected cell lines derived from kidney cancers express MDR1 RNA and are multidrug-resistant, and this resistance can be reversed by verapamil and quinidine.

There is a great need for compositions and methods for inhibiting the multidrug resistance function of the P-protein during chemotherapy treatment of cancers so that the cytotoxic drugs administered to patients are not excluded from the cancer cell. The compositions and methods should not be toxic to other cells or tissues, and should specifically inhibit the ability of P-glycoprotein to confer multidrug resistance on cells.

Summary of the Invention

The present invention generally involves methods and compositions for reducing or mitigating multidrug resistance in cancer cells and tissues. The present invention more particularly involves antigenic P-glycoprotein-containing compositions that, when administered to a human or animal with a multidrug resistance cancer or tumor, elicit the production of antibodies specific for certain portions of the P-glycoprotein molecule which reduce multidrug resistance, thus making the cancer or tumor more sensitive to anti-cancer drugs.

Examples of P-glycoprotein-containing compositions include liposomes having portions of P-glycoprotein externally presented on their surfaces, P-protein peptide fragments, modified P-protein fragments and liposomes containing P-protein peptide fragments and/or modified P-protein peptide fragments. The present invention also encompasses mixtures of at least one adjuvant and at least one of the above identified P-glycoprotein-containing compositions. It is contemplated that one or more of the aforementioned compositions can be used as a vaccine to reduce multidrug resistance in cancer cells in a human or animal with the cancer.

The present invention also includes antibodies specific for externally accessible regions or epitopes of the P-protein. For example, these antibodies would be produced by and purified from humans with strong immune systems, and injected into patients with weak or non-functional immune systems in need of such circulating antibodies. Thus, according to the present invention, multidrug resistance in cells and tissues can be reduced either by active immunization of an individual having multidrug resistance using antigenic P-glycoprotein-containing compositions or by passive immunization via administering an antibody or a group of antibodies specific for P-protein epitopes.

Accordingly, it is an object of the present invention to provide methods and compositions for reducing multidrug resistance in a human or animal with a multidrug resistant cancer and cells and tissues having multidrug resistance.

It is another object of the present invention to provide methods and compositions for reducing multidrug resistance in cancer cells.

It is yet another object of the present invention to provide methods and compositions for vaccinating a human or animal against certain extracellular sequences of P-protein to reduce the multidrug resistance of cells and tissues.

It is yet another object of the present invention to provide methods and compositions for passively immunizing a human or animal against certain extracellular sequences of P-protein to reduce the multidrug resistance of cells and tissues.

Another object of the present invention is to provide P-glycoprotein-containing compositions that are antigenic and elicit an immune response against P-glycoprotein.

It is still another object of the present invention to provide P-protein peptide fragments corresponding to the externally exposed portions of P-protein and methods of use thereof.

Yet another object of the present invention is to provide P-protein peptide fragments modified with antigenic carriers to increase the antigenic response to P-protein peptide fragments, and methods of use thereof.

It is yet another object of the present invention to provide P-protein peptide fragments and modified P-protein peptide fragments in liposomes and method of use thereof.

It is still another object of the present invention to provide P-protein peptide fragment-containing compositions in combination with adjuvants to stimulate the immune response.

Another object of the present invention is antibodies specific to externally exposed regions of P-glycoprotein useful for passively immunizing a human or animal and thereby reducing multidrug resistance.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiment and the appended claims.

Brief Description of the Figures

Figure 1 is a schematic representation of the putative structure of P-protein and its orientation in the membrane of a liposome.

Figure 2 represents a P-protein peptide fragment modified at one end with a lipophilic moiety and inserted into a lipid bilayer resulting in a "tail presentation."

Figure 3 represents a P-protein peptide fragment modified at both ends with lipophilic moieties and inserted into a lipid bilayer resulting in a "loop presentation."

Figure 4 is a graph illustrating the percent cells surviving as a function of accumulation in the cells of doxorubicin.

Figure 5 is a histogram illustrating the level of fluorescence intensity, indicating the level of anti-P-protein peptide binding, as a function of exposure to various doxorubicin concentrations.

Figure 6A is a dot blot determination in which antibodies directed against the liposome carrier alone were incubated with the 38-mer, 16-mer and 14-mer mouse P-protein peptide fragments, SEQ. ID. NOS: 1, 2, and 4, respectively.

Figure 6B is a dot blot determination in which antibodies directed against the liposome carrier alone were incubated with the 38-mer, 16-mer and 14-mer mouse P-protein peptide fragments, SEQ. ID. NOS: 1, 2, and 4, respectively.

Figure 7A is a dot determination in which antibodies directed against a mixture of P-protein peptide fragments (38-mer, 16-mer, 9-mer, and 14-mer; SEQ. ID. NOS: 1-4,

respectively) in liposomes are incubated with 38-mer, 16-mer, and 14-mer P-protein peptide fragments, SEQ. ID. NOS: 1, 2, and 4, respectively.

Figure 7B is a dot blot determination in which antibodies directed against a mixture of P-protein peptide fragments (38-mer, 16-mer, 9-mer, and 14-mer; SEQ. ID. NOS: 1-4, respectively) in liposomes are incubated with 38-mer, 16-mer, and 14-mer P-protein peptide fragments, SEQ. ID. NOS: 1, 2, and 4, respectively.

Detailed Description of the Present Invention

The present invention comprises methods and compositions for reducing multidrug resistance in a human or animal. The antigenic P-glycoprotein-containing compositions of the present invention include, but are not limited to, P-glycoprotein containing liposomes, P-protein peptide fragments, P-protein peptide fragments combined with adjuvants, modified P-protein peptide fragments, modified P-protein peptide fragments combined with adjuvants, liposomes containing P-protein peptide fragments, and liposomes containing modified P-protein peptide fragments. Still further, the present invention includes antibodies directed against, and specific for, the externally accessible portions of P-glycoprotein.

As used herein, the term "P-glycoprotein" refers to a membrane bound glycosylated protein having amino acid sequences externally exposed on the outer surface of cells which confers multidrug resistance to those cells. The term "P-protein" refers to the amino acid sequence of P-glycoprotein without carbohydrate groups attached. This term "P-protein peptide" refers to incomplete amino acid sequences of P-protein or peptide fragments of P-protein. Also as used herein, the term "antigenic carrier" refers to substances which when bound to P-glycoprotein, P-protein, P-protein fragments, or modified P-

protein peptide fragments, elicit or enhance an immune response against P-glycoprotein in humans or animals. Further, the term "effective amount" refers to the amount of P-glycoprotein, P-protein, P-protein fragments, or modified P-protein peptide fragments, which when administered to a human or animal causes a reduction in multidrug resistance and increased sensitivity to chemotherapeutic agents. The effective amount can be readily determined by one of skill in the art following routine procedures. For example, P-peptide compositions may be administered in a range of approximately 100 ug to 1 mg per patient, though this range is not intended to be limiting. This approximate range of administration has been used, e.g., for peptide vaccines for malaria. The actual amount of P-glycoprotein composition required to elicit an immune response will vary for each individual patient depending on the antigenicity of the P-glycoprotein composition administered and on the immune response of the individual. Consequently, the specific amount administered to an individual will be determined by routine experimentation and based upon the experience of one skilled in the art.

As shown in Fig. 1, mouse P-glycoprotein exposes on the surface of a liposome six peptide loops having sequences of 9 to 38 amino acids each. Human P-glycoprotein has 5 externally exposed peptide loops. The amino acid sequences of these externally exposed loops are an essential feature of the invention. These sequences are incorporated into the P-protein-containing compositions of the present invention, which may be used to directly immunize a human or animal having a cancer or tumor that is resistant to many drugs. Despite the presence of P-glycoprotein on certain normal cell types, e.g. kidney and liver cells as discussed above, induced autoimmunity against P-glycoprotein is not harmful.

Alternatively, the P-glycoprotein containing compositions of the present invention may be used to produce antibodies directed against externally exposed regions or epitopes of P-protein. These antibodies can be administered to multidrug resistant individuals to passively immunize them against P-glycoprotein and thereby reduce multidrug resistance.

More specifically, the present invention encompasses P-glycoprotein inserted into liposomes so as to present on the liposome surface portions of P-glycoprotein normally exposed on cell surfaces. Such liposomes may be made by reconstituting liposomes in the presence of purified or partially purified P-glycoprotein. Additionally, P-protein peptide fragments that correspond to externally presented loops of P-protein, i.e. those portions of the P-protein presented on the outside of cell surfaces and accessible to antibodies are encompassed by the present invention. The present invention also includes P-protein peptide fragments modified so as to increase their antigenicity, for example by the attachment of antigenic carriers, and adjuvant mixtures thereof. Examples of adjuvants include, but are not limited to, lipophilic muramyl dipeptide derivatives, nonionic block polymers, aluminum hydroxide or aluminum phosphate adjuvant, and mixtures thereof.

The present invention further encompasses P-protein fragments modified with lipophilic moieties, such as palmitic acid, that facilitate insertion into liposomes so that the P-protein orients itself in the liposome membrane in a manner similar to its orientation in natural membranes. Lipophilic moieties of the present invention may be fatty acids, triglycerides and phospholipids wherein the fatty acid carbon back bones have at least 10 carbon atoms. Most preferable are lipophilic moieties having fatty acids with a carbon backbone of at least approximately 14 carbon atoms and up to approximately 24 carbon atoms. The most preferred lipophilic moieties have a

carbon backbone of at least 14 carbon atoms. Examples of lipophilic moieties include, but are not limited to, palmitic acid, stearic acid, myristic acid, lauric acid, oleic acid, linoleic acid, and linolenic acid. The most preferred lipophilic moiety is palmitic acid.

As shown in Fig. 2, a P-protein peptide fragment (**10**) may be modified by coupling to it a lipophilic moiety (**12**) which facilitates insertion of the molecule into a liposome membrane (**14**) resulting in a "tail presentation." Another possible modified P-protein peptide fragment is depicted in Fig. 3, where a peptide fragment (**16**) is modified at both ends of the amino acid sequence with lipophilic moieties (**18**), which can be either the same or different moieties, so as to facilitate insertion into a liposome membrane (**20**) resulting in a "loop presentation."

P-protein peptide fragments and modified P-protein peptide fragments, and adjuvant mixtures thereof, as well as the above-described liposome compositions can be administered to a human or animal to induce immunity to certain regions of P-glycoprotein thereby reducing that individual's multidrug resistance. This induced autoimmunity is not deleterious to healthy individuals, as shown in Table 2. After the human or animal has been immunized against the P-protein, the human or animal will have circulating antibodies against P-protein which bind to the externally exposed portions of P-protein, thereby reducing or inactivating its ability to confer multidrug resistance on the cell.

TABLE 2

BLOOD COUNT OF MICE IMMUNIZED AGAINST P-GLYCOPROTEIN SEGMENTS

Cell Type	Control Non-Injected	Liposome-Injected	Liposome-Peptide Injected

WBC	8900±854.40	8600±556.78	8600±1228.82
NE	23%±3.00	23.66%±3.79	21.66%± 2.08
LY	71%±2.00	74.33%±2.31	72.66%±2.52
MO	66%±1.00	4.33%± 0.58	5%±1.00
EO	0.66%±0.58	1%±0.00	0.66%±0.58
BA	0%±0.00	0%±0.00	0%±0.00

Where WBC is white blood cells; NE is neutriphile cells; LY is lymphocte cells; MO is monocyte cells; EO is eosinocyte cells; and BA is basophile cells.

Liposomes with the P-glycoprotein inserted into the membrane, as well as other antigenic compositions containing one or more P-protein peptide fragments, also can be used to produce a panel of monoclonal or polyclonal antibodies that are specific for the externally exposed portions of the P-protein, which can be used to inhibit multidrug resistance. Antibodies can be made by methods that are well known to those of ordinary skill in the art, as described, for example, in Alving, C.R. et al. *Proc. Nat'l. Acad. Sci. USA* (1992) 89:358-362, and Alving, C.R., *J. Immuno. Meth.* (1991) 140:1-13, hereby incorporated by reference. These antibodies, when administered to multidrug resistant individuals, will bind the extracellular portions of the P-glycoprotein and are capable of inhibiting the activity of P-glycoprotein, thereby reducing multidrug resistance in a human or animal. It is to be understood that the present invention can be practiced using P-protein from any source.

The anti-P-protein antibodies can be administered to a human or animal by any appropriate means, preferably by injection, to reduce the multidrug resistance activity of P-glycoprotein. For example, a patient undergoing chemotherapy for a cancer diagnosed as being multidrug resistant, or developing multidrug resistance, may be treated with P-glycoprotein reconstituted in liposomes or with antigenic peptide fragments thereof to produce circulating anti-P-protein antibodies.

Alternatively, a anti-P-protein antibody preparation can be administered to the patient preferably before the administration of the chemotherapeutic drug or drugs. Whether internally produced or provided from external sources, the circulating antibodies will bind to the exposed portions of the P-glycoprotein and reduce or inactivate its ability to exclude drugs from the cell.

Briefly described, the gene or genes encoding the human multidrug transport system are cloned from human multidrug-resistant KB cells by selecting for resistance to high levels of colchicine, vinblastine, or doxorubicin (Adriamycin). These cells contain many minute and double minute chromosomes, and by ingel renaturation analysis, amplified DNA segments common to all these cell lines are identified in their genomic DNA. By homology with similarly amplified sequences in multidrug-resistant Chinese hamster cells, two related human genomic sequences are isolated from multidrug-resistant KB cells. These were designated MDR1 and MDR2. MDR1 and MDR2 are genetically linked and map to chromosome 7q.

Expression of a 4.5-kilobase mRNA, corresponding to the MDR1 gene only, is found to correlate with drug resistance in many different multidrug resistant cells. Using the genomic MDRl probe, overlapping cDNA segments for the entire MDR1 RNA is obtained and sequenced. This full-length cDNA encodes the cell surface 170,000-Dalton P-protein. Similar MDR cDNAs have been cloned and completely sequenced from the mouse. Based on over expression of the MDR RNA in multidrug-resistant cells, cDNA clones from the mouse and hamster can also be obtained.

Liposomes that can be used in the compositions of the present invention include all those known to one skilled in the art. Any of the standard lipids useful for making liposomes may be used. Monolayer, bilayer and multi-layer liposomes may be used to make compositions of the present invention. While any method

of making liposomes known to one skilled in the art may be used, the most preferred liposomes are made according to the method of Alving et al., *Infect. Immun.* 60:2438-2444, 1992, hereby incorporated by reference. The liposome can optionally contain a detoxified Lipid A such as Monophosphoryl lipid A (Ribi Immunochem. Hamilton Montana).

This invention is further illustrated by the following examples, which are not construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

Example I

Expression of P-glycoprotein in the baculovirus-insect cell system

A full-length mdr c-DNA (clone MDRl 1) is obtained from Dr. Housman. The baculovirus expression vector is constructed as described in Webb, N.R. et al. (1989) *Proc. Natl. Acad. Sci. USA* 86, 7731-7735, hereby incorporated by reference. A recombinant baculovirus (Ac-P) containing a cDNA encoding full-length P-protein under transcriptional regulation of the polyhedrin promoter is produced by co-transfecting pAc-P DNA with wild type *Autographa californica* nuclear polyhedrosis virus (AcMNPV) DNA by calcium phosphate precipitation. The occlusion-negative virus will be plaque-purified and propagated in *Spodoptera frugiperda 9* cells.

Example II

The resulting virus is used to infect Sf-9 cells in air-lift fermentors as described in Webb, N.R. et al. (1989) *Proc. Natl. Acad. Sci. USA* 86, 7731-7735. Extraction and purification of the P-protein is performed as described in Webb, et al. The

Sf-9 cells expressing the P-protein is subjected to flow cytometry analysis using an FITC-stained goat anti-mouse antibody and a monoclonal antibody against the protein. The purified P-protein, assayed by gel electrophoresis and immunoblotting, is electroinserted (Mouneime, Y., et al., 1990, *Biochem.*, hereby incorporated by reference) in large liposomes containing lipid A and injected i.m. to mice. Antibodies elicited against P-protein are determined by ELISA, using the recombinant protein and horse-radish peroxidase-conjugated goat anti-mouse immunoglobulins.

P-protein peptide fragments, representing the externally located extracellular regions of P-protein in mice and humans have been synthesized and are shown in Table 3.

Table 3

A. *Mouse* Peptide Sequences

1 Gly Asn Met Thr Asp Ser Phe Thr Lys Ala Glu Ala Ser Ile Leu Pro
1 5 10 15

Ser Ile Thr Asn Gln Ser Gly Pro Asn Ser Thr Leu Ile Ile Ser Asn
20 25 30

Ser Ser Leu Glu Glu Glu
35

2 Lys Val Leu Thr Ser Phe Thr Asn Lys Glu Leu Gln Ala Tyr Ala Lys
1 5 10 15

3 Leu Ser Asn Glu Tyr Ser Ile Gly Glu
1 5

4 Ser Arg Asp Asp Asp Met Glu Thr Lys Arg Gln Asn Glu Asn
1 5 10

B. *Human* Peptide Sequences

5 Gly Glu Met Thr Asp Ile Phe Ala Asn Ala Gly Asn Leu Glu Asp
1 5 10 15

Leu Met Ser Asn Ile Thr Asn Arg Ser Asp Ile Asn Asp Thr Gly Phe
20 25 30

Phe Met Asn Leu Glu Glu Asp Met Thr Arg Tyr Ala Tyr Tyr Tyr Ser
35 40 45

6 Arg Gly Trp Lys Leu Thr
5

7 Thr Thr Leu Val Leu Ser Gly Glu
5

8 Arg Phe Gly Ala Tyr Leu Val Ala His Lys Leu Met Ser Phe Glu Asp
1 5 10 15

9 Phe Ser Lys Ile Ile Gly Val Phe Thr Arg Ile Asp Asp Pro Glu Thr
1 5 10 15

Lys Arg Gln Asn Ser Asn Leu Phe Ser
20 25

It is believed that the most preferred peptide fragments for eliciting an immune response and reducing multidrug resistance are the 14 and 16 amino acid mouse sequences indicated in Table 2, SEQ. ID. NOs: 4 and 2, respectively.

The peptide fragments can be further modified, at either the N- and C- terminus, or both, by addition of carriers or other moieties that render the P-protein peptide more antigenic and by lipophilic moieties such as palmitic acid using standard methods well known in the art.

Example III

Sera containing the anti-P-protein antibodies are produced and used in vitro to sensitize mdr L1210 and KD cells to adriamycine, vincristine, doxorubicin and other chemotherapy drugs. Mice are inoculated with the mdr mice leukemia L1210 cells. After developing the disease, a group of the sick mice is immunized with P-protein-liposomes-lipid A and their survival after treatment with chemotherapy agents compared to that of the positive control group, subjected to the same chemotherapy.

For example, antibodies directed against a mixture of all four mouse P-protein peptide fragments (SEQ. ID NOS: 1-4), modified with liposomes inserted in the "loop presentation" as shown in Figure 3 (antipeptide), and antibodies directed against liposomes not containing P-protein peptide fragments (antiliposomes) were raised in mice.

Table 4 shows an exemplary liposome composition used to immunize mice.

Table 4: Composition of the Liposome-Peptide Antigen

A-Lipids	μg/inoculation
DMPC	628
DMPG	70
Cholesterol	262
Lipid A	40
+	
Alum	180
B-Peptides	
38 mer	21
16 mer	9
14 mer	8
9 mer	5

Multidrug resistant L1210 cells were then incubated with and without the antibodies and subsequently challenged with the chemotherapy drug doxorubicin. Normal L1210 cells were used as an additional control. It was found that cells treated with antipeptide antibody became more sensitive to the drug, as illustrated in Table 5.

Table 5.

Cells	% Survival	LD_{50}
Normal L1210	49	$4 \times 10^{-6}M$
MDR L1210	62	$4 \times 10^{-5}M$
MDR L1210 + antiliposome	61	$4 \times 10^{-5}M$
MDR L1210 + antipeptide	40	$4 \times 10^{-7}M$

Figure 4 illustrates the finding that multidrug resistant L1210 cells treated with increasing titers of anti-P-protein antibody accumulate increased levels of doxorubicin and have a correspondingly decreased survival percentage. Similarly, Figure 5 illustrates the finding that there is a correlation between binding of anti-P-protein antibody to MDR L1210 cells and the accumulation within the cells of doxorubicin. The amount of antibody bound to, and doxorubicin accumulated within, the cells was measured by fluorescence. Controls using antibodies directed only against the liposomal carrier showed high antibody binding to the cells without a corresponding increase in doxorubicin accumulation. These results demonstrate that anti-P-protein antibodies are effective in decreasing multidrug resistance.

An added advantage of the invention apparent from the results presented in Table 3 is the increased sensitivity, relative to normal cells, of MDR cells treated with antipeptide. The results demonstrate that MDR cells treated with antipeptide not only lose their multidrug resistance, but may become even more sensitive to the drug than normal cells. The percent survival of treated MDR cells is only 40%, well below that of normal cells (49%), and the LD_{50} of treated MDR cells is a full order of magnitude lower than normal cells as well. Thus, treatment of cancers and tumors with compositions of the present invention may permit selective and preferential targeting of otherwise inherently drug resistant cells, resulting in greatly increased efficacy of chemotherapy treatment.

The preferred P-protein peptide fragments are

Lys Val Leu Thr Ser Phe Thr Asn Lys Glu Leu Gln Ala Tyr Ala Lys
1 5 10 15

and

Ser Arg Asp Asp Asp Met Glu Thr Lys Arg Gln Asn Glu Asn
1 5 10

SEQ. ID. NOS: 2 and 4, respectively.

Figures **6a** and **6b** represent duplicate dot blot determinations in which antibodies directed against the liposome carrier alone were incubated with the 38-mer, 16-mer and 14-mer mouse P-protein peptide fragments, SEQ. ID. NOs: 1, 2 and 4, respectively.

Sample #	**Description**
1A, 5D	100 ng peptide 38 mer + antiliposome serum dil 1/200
1B, 5E	10 ng peptide + antiliposome serum dil 1/200
1C, 5F	1 ng peptide 38 mer + antiliposome serum dil 1/200
2A, 6D	100 ng peptide 16 mer + antiliposome serum dil 1/200
2B, 6E	10 ng peptide 16 mer + antiliposome serum dil 1/200
2C, 6F	1 ng peptide 16 mer + antiliposome serum dil 1/200
3A, 7D	100 ng peptide 14 mer + antiliposome serum dil 1/200
3B, 7E	10 ng peptide 14 mer + antiliposome serum dil 1/200
3C, 7F	1 ng peptide 14 mer + antiliposome serum dil 1/200
4A, 8D	GAM PE and 1000 ng mouse Mab C6
4B, 8E	100 ng mouse Mab C6
4C, 8F	10 ng mouse Mab C6 and GAM AP

In contrast, Figures **7a** and **7b**, which represent duplicate dot blot determinations in which antibodies directed against a mixture of P-protein peptide fragments (38-mer, 16-mer, 9-mer and 14-mer; SEQ. ID. NOS: 1-4, respectively) in liposomes are incubated with 38-mer, 16-mer and 14-mer P-protein peptide fragments, SEQ. ID. NOS: 1, 2 and 4, respectively.

Sample #	Description
1A, 5D	100 ng peptide 38 mer + antipeptide serum dil 1/200
1B, 5E	10 ng peptide 38 mer + antipeptide serum dil 1/200
1C, 5F	1 ng peptide 38 mer + antipeptide serum dil 1/200
2A, 6D	100 ng peptide 16 mer + antipeptide serum dil 1/200
2B, 6E	10 ng peptide 16 mer + antipeptide serum dil 1/200
2C, 6F	1 ng peptide 16 mer + antipeptide serum dil 1/200
3A, 7D	100 ng peptide 14 mer + antipeptide serum dil 1/200
3B,7E	10 ng peptide 14 mer + antipeptide serum dil 1/200
3C, 7F	1 ng peptide 14 mer + antipeptide serum dil 1/200
4A, 8E	1000 ng mouse Mab C6
4B, 8D	GAM AP and 100 ng mouse Mab C6
4C, 8F	GAM PE and 10 ng mouse Mab C6

As can be seen the antipeptide serum reacts with the mouse 16-mer and 14-mer, SEQ. ID. NOS: 2 and 4, respectively.

While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without departing from the spirit and scope thereof.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Nicolau, Yves Claude,
Tosi, Pierre-Francois

(ii) TITLE OF INVENTION: Method For Reducing Multidrug Resistance in Cells and Tissues

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Jones & Askew
(B) STREET: 191 Peachtree Street, 37th Floor
(C) CITY: Atlanta
(D) STATE: Georgia
(E) COUNTRY: USA
(F) ZIP: 30303-1769

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 3.50
(B) COMPUTER: MacIntosh
(C) OPERATING SYSTEM: 7.0
(D) SOFTWARE: Microsoft Word

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 07/970,416
(B) FILING DATE: January 13, 1993
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Larry W. Stults
(B) REGISTRATION: 34,025
(C) REFERENCE/DOCKET NUMBER: 05213-0020

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE 404-818-3700
(B) TELEFAX: 404-818-3799

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38
(B) TYPE: peptide
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Gly Asn Met Thr Asp Ser Phe Thr Lys Ala Glu Ala Ser Ile Leu Pro

```
1               5                   10                  15
Ser Ile Thr Asn Gln Ser Gly Pro Asn Ser Thr Leu Ile Ile Ser Asn
            20                  25                  30
Ser Ser Leu Glu Glu Glu
        35

         (3)   INFORMATION FOR SEQ ID NO: 2:
(i)            SEQUENCE CHARACTERISTICS:
         (A)   LENGTH: 16
         (B)   TYPE: peptide
         (D)   TOPOLOGY:  linear
(xi)     SEQUENCE DESCRIPTION:  SEQ ID NO: 2:

Lys Val Leu Thr Ser Phe Thr Asn Lys Glu Leu Gln Ala Tyr Ala Lys
1               5                   10                  15

         (4)   INFORMATION FOR SEQ ID NO: 3:
(i)            SEQUENCE CHARACTERISTICS:
         (A)        LENGTH: 9
         (B)   TYPE: peptide
         (D)   TOPOLOGY:  linear
(xi)     SEQUENCE DESCRIPTION:  SEQ ID NO: 3:

Leu Ser Asn Glu Tyr Ser Ile Gly Glu
1               5

         (5)   INFORMATION FOR SEQ ID NO: 4:
(i)            SEQUENCE CHARACTERISTICS:
         (A)   LENGTH: 14
         (B)   TYPE: peptide
         (D)   TOPOLOGY:  linear
(xi)     SEQUENCE DESCRIPTION:  SEQ ID NO: 4:

Ser Arg Asp Asp Asp Met Glu Thr Lys Arg Gln Asn Glu Asn
1               5                   10

         (6)   INFORMATION FOR SEQ ID NO: 5:
(i)            SEQUENCE CHARACTERISTICS:
         (A)   LENGTH: 47
         (B)   TYPE: peptide
         (D)   TOPOLOGY:  linear
(xi)     SEQUENCE DESCRIPTION:  SEQ ID NO: 5:

Gly Glu Met Thr Asp Ile Phe Ala Asn Ala Gly Asn Leu Glu Asp Leu
1               5                   10                  15
```

Met Ser Asn Ile Thr Asn Arg Ser Asp Ile Asn Asp Thr Gly Phe Phe
20 25 30

Met Asn Leu Glu Glu Asp Met Thr Arg Tyr Ala Tyr Tyr Tyr Ser
35 40 45

(7) INFORMATION FOR SEQ ID NO: 6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6
(B) TYPE: peptide
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Arg Gly Trp Lys Leu Thr
1 5

(8) INFORMATION FOR SEQ ID NO: 7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8
(B) TYPE: peptide
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Thr Thr Leu Val Leu Ser Gly Glu
1 5

(9) INFORMATION FOR SEQ ID NO: 8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16
(B) TYPE: peptide
(C) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Arg Phe Gly Ala Tyr Leu Val Ala His Lys Leu Met Ser Phe Glu Asp
1 5 10 15

(10) INFORMATION FOR SEQ ID NO: 9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25
(B) TYPE: peptide
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Phe Ser Lys Ile Ile Gly Val Phe Thr Arg Ile Asp Asp Pro Glu Thr
1 5 10 15

Lys Arg Gln Asn Ser Asn Leu Phe Ser
20 25

Claims:

1. A composition comprising,

P-glycoprotein inserted in a liposome wherein one or more of the portions of the P-glycoprotein molecule that are exposed on the outer surface of cells are externally exposed on the surface of the liposome.

2. The composition of claim 1, wherein the externally exposed portions of the P-glycoprotein are selected from the group consisting of

Gly Asn Met Thr Asp Ser Phe Thr Lys Ala Glu Ala Ser Ile Leu Pro
1 5 10 15

Ser Ile Thr Asn Gln Ser Gly Pro Asn Ser Thr Leu Ile Ile Ser Asn
20 25 30

Ser Ser Leu Glu Glu Glu
35

SEQ ID. NO: 1;

Lys Val Leu Thr Ser Phe Thr Asn Lys Glu Leu Gln Ala Tyr Ala Lys
1 5 10 15

SEQ ID. NO: 2;

Leu Ser Asn Glu Tyr Ser Ile Gly Glu
1 5

SEQ ID. NO: 3;

Ser Arg Asp Asp Asp Met Glu Thr Lys Arg Gln Asn Glu Asn
1 5 10

SEQ ID. NO: 4;

Gly Glu Met Thr Asp Ile Phe Ala Asn Ala Gly Asn Leu Glu Asp
1 5 10 15

Leu Met Ser Asn Ile Thr Asn Arg Ser Asp Ile Asn Asp Thr Gly Phe
20 25 30

Phe Met Asn Leu Glu Glu Asp Met Thr Arg Tyr Ala Tyr Tyr Tyr Ser
35 40 45

SEQ ID. NO: 5;

Arg Gly Trp Lys Leu Thr
5

SEQ ID. NO: 6;

Thr Thr Leu Val Leu Ser Gly Glu

SEQ ID. NO: 7;

Arg Phe Gly Ala Tyr Leu Val Ala His Lys Leu Met Ser Phe Glu Asp
1 5 10 15

SEQ ID. NO: 8; AND

Phe Ser Lys Ile Ile Gly Val Phe Thr Arg Ile Asp Asp Pro Glu Thr
1 5 10 15

Lys Arg Gln Asn Ser Asn Leu Phe Ser
20 25

SEQ ID. NO: 9.

3. The composition of Claim 1, wherein at least

Lys Val Leu Thr Ser Phe Thr Asn Lys Glu Leu Gln Ala Tyr Ala Lys
1 5 10 15

(SEQ ID. NO. 2) is exposed on the surface of the liposome.

4. The composition of Claim 1, wherein at least

Ser Arg Asp Asp Asp Met Glu Thr Lys Arg Gln Asn Glu Asn
1 5 10

(SEQ ID. NO. 4) is exposed on the surface of the liposome.

5. The composition of claim 1, further comprising an adjuvant.

6. The composition of claim 5, wherein the adjuvant is selected from the group consisting of lipophilic muramyl dipeptide derivatives, nonionic block polymers, aluminum hydroxide, aluminum phosphate and Lipid A.

7. The composition of Claim 5, wherein the adjuvant is Lipid A.

8. A composition comprising,

an amino acid sequence which corresponds to an amino acid sequence of P-glycoprotein that is externally exposed on the surface of cells.

9. The composition of Claim 8, wherein the amino acid sequence is selected from the group consisting of:

SEQ ID. NO: 1:

Gly Asn Met Thr Asp Ser Phe Thr Lys Ala Glu Ala Ser Ile Leu Pro
1 5 10 15

Ser Ile Thr Asn Gln Ser Gly Pro Asn Ser Thr Leu Ile Ile Ser Asn
20 25 30

Ser Ser Leu Glu Glu Glu
35

SEQ ID. NO: 2:
Lys Val Leu Thr Ser Phe Thr Asn Lys Glu Leu Gln Ala Tyr Ala Lys
1 5 10 15

SEQ ID. NO: 3:
Leu Ser Asn Glu Tyr Ser Ile Gly Glu
1 5

SEQ ID. NO: 4:
Ser Arg Asp Asp Asp Met Glu Thr Lys Arg Gln Asn Glu Asn
1 5 10

SEQ ID. NO: 5:

Gly Glu Met Thr Asp Ile Phe Ala Asn Ala Gly Asn Leu Glu Asp
1 5 10 15

Leu Met Ser Asn Ile Thr Asn Arg Ser Asp Ile Asn Asp Thr Gly Phe
20 25 30

Phe Met Asn Leu Glu Glu Asp Met Thr Arg Tyr Ala Tyr Tyr Tyr Ser
35 40 45

SEQ ID. NO: 6:

Arg Gly Trp Lys Leu Thr

SEQ ID. NO: 7:

Thr Thr Leu Val Leu Ser Gly Glu
5

SEQ ID. NO: 8:

Arg Phe Gly Ala Tyr Leu Val Ala His Lys Leu Met Ser Phe Glu Asp
1 5 10 15

SEQ ID. NO: 9:
Phe Ser Lys Ile Ile Gly Val Phe Thr Arg Ile Asp Asp Pro Glu Thr
1 5 10 15

Lys Arg Gln Asn Ser Asn Leu Phe Ser
20 25

10. The composition of Claim 8, wherein the amino acid sequence is:

Lys Val Leu Thr Ser Phe Thr Asn Lys Glu Leu Gln Ala Tyr Ala Lys
1 5 10 15

(SEQ ID. NO. 2).

11. The composition of Claim 8, wherein the amino acid sequence is:

Ser Arg Asp Asp Asp Met Glu Thr Lys Arg Gln Asn Glu Asn
1 5 10

(Seq ID. No. 4).

12. The composition of claim 8, further comprising an adjuvant.

13. The composition of claim 12, wherein the adjuvant is selected from the group consisting of lipophilic

muramyl dipeptide derivatives, nonionic block polymers, aluminum hydroxide, aluminum phosphate and Lipid A.

14. The composition of Claim 12, wherein the adjuvant is Lipid A.

15. The composition of Claim 8, further comprising an antigenic carrier attached to the amino acid sequence so as to elicit an immune response against the amino acid sequence when administered to a human or animal.

16. The composition of Claim 8, further comprising at least one lipophilic moiety attached to the amino acid sequence and a liposome, wherein the lipophilic moiety is inserted into the liposome such that the amino acid sequence is exposed on the external surface of the liposome.

17. The composition of Claim 16, wherein the lipophilic moiety comprises at least one long chain fatty acid having at least 10 carbon atoms in the lipid backbone.

18. The composition of Claim 17, wherein the lipophilic moiety is selected from the group consisting of palmitic acid, stearic acid, myristic acid, lauric acid, oleic acid, linoleic acid, and linolenic acid.

19. The composition of Claim 18 wherein the lipophilic moiety is palmitic acid.

20. The composition of claim 16, further comprising an adjuvant.

21. The composition of claim 20, wherein the adjuvant is selected from the group consisting of lipophilic muramyl dipeptide derivatives, nonionic block polymers, aluminum hydroxide, aluminum phosphate and Lipid A

22. The composition of Claim 21, wherein the adjuvant is Lipid A.

23. A method for reducing multidrug resistance in a human or animal comprising,

administering to a human or animal having multidrug resistance an effective amount of a P-glycoprotein-containing liposome composition, wherein the P-glycoprotein is inserted into the liposome membrane such that one or more of the portions of the P-glycoprotein molecule that are exposed on the outer surface of cells are externally exposed on the outer surface of the liposome.

24. The method of claim 23, wherein said externally exposed portion of the P-glycoprotein is selected from the group consisting of:

Gly Asn Met Thr Asp Ser Phe Thr Lys Ala Glu Ala Ser Ile Leu Pro
1 5 10 15

Ser Ile Thr Asn Gln Ser Gly Pro Asn Ser Thr Leu Ile Ile Ser Asn
20 25 30

Ser Ser Leu Glu Glu Glu
35

SEQ ID. NO: 1;

Lys Val Leu Thr Ser Phe Thr Asn Lys Glu Leu Gln Ala Tyr Ala Lys
1 5 10 15

SEQ ID. NO: 2;

Leu Ser Asn Glu Tyr Ser Ile Gly Glu
1 5

SEQ ID. NO: 3;

Ser Arg Asp Asp Asp Met Glu Thr Lys Arg Gln Asn Glu Asn
1 5 10

SEQ ID. NO: 4;

Gly Glu Met Thr Asp Ile Phe Ala Asn Ala Gly Asn Leu Glu Asp
1 5 10 15

Leu Met Ser Asn Ile Thr Asn Arg Ser Asp Ile Asn Asp Thr Gly Phe
20 25 30

Phe Met Asn Leu Glu Glu Asp Met Thr Arg Tyr Ala Tyr Tyr Tyr Ser
35 40 45

SEQ ID. NO: 5;

Arg Gly Trp Lys Leu Thr
5

SEQ ID. NO: 6;

Thr Thr Leu Val Leu Ser Gly Glu
5

SEQ ID. NO: 7;

Arg Phe Gly Ala Tyr Leu Val Ala His Lys Leu Met Ser Phe Glu Asp
1 5 10 15

SEQ ID. NO: 8; AND

Phe Ser Lys Ile Ile Gly Val Phe Thr Arg Ile Asp Asp Pro Glu Thr
1 5 10 15

Lys Arg Gln Asn Ser Asn Leu Phe Ser
20 25

SEQ ID. NO: 9.

25. The method of Claim 24, wherein at least

Lys Val Leu Thr Ser Phe Thr Asn Lys Glu Leu Gln Ala Tyr Ala Lys
1 5 10 15

(SEQ ID. NO. 2) is exposed on the surface of the liposome.

26. The method of Claim 24, wherein at least is

Ser Arg Asp Asp Asp Met Glu Thr Lys Arg Gln Asn Glu Asn

1 5 10
(SEQ ID. NO. 4) exposed on the surface of the liposome.

27. The method of claim 23, further comprising an adjuvant.

28. The method of claim 27, wherein the adjuvant is selected from the group consisting of lipophilic muramyl dipeptide derivatives, nonionic block polymers, aluminum hydroxide, aluminum phosphate and Lipid A.

29. The composition of Claim 28, wherein the adjuvant is Lipid A.

30. A method for reducing multidrug resistance in a human or animal comprising,

administering to a human or animal having multidrug resistance an effective amount of an amino acid sequence corresponding to an amino acid sequence of P-glycoprotein that is externally exposed on the surface of cells.

31. The method of Claim 30, wherein the amino acid sequence is selected from the group consisting of

Gly Asn Met Thr Asp Ser Phe Thr Lys Ala Glu Ala Ser Ile Leu Pro
1 5 10 15

Ser Ile Thr Asn Gln Ser Gly Pro Asn Ser Thr Leu Ile Ile Ser Asn
20 25 30

Ser Ser Leu Glu Glu Glu
35

SEQ ID. NO: 2:
Lys Val Leu Thr Ser Phe Thr Asn Lys Glu Leu Gln Ala Tyr Ala Lys
1 5 10 15

SEQ ID. NO: 3:
Leu Ser Asn Glu Tyr Ser Ile Gly Glu

1 5

SEQ ID. NO: 4:
Ser Arg Asp Asp Asp Met Glu Thr Lys Arg Gln Asn Glu Asn
1 5 10

SEQ ID. NO: 5:

Gly Glu Met Thr Asp Ile Phe Ala Asn Ala Gly Asn Leu Glu Asp
1 5 10 15

Leu Met Ser Asn Ile Thr Asn Arg Ser Asp Ile Asn Asp Thr Gly Phe
20 25 30

Phe Met Asn Leu Glu Glu Asp Met Thr Arg Tyr Ala Tyr Tyr Tyr Ser
35 40 45

SEQ ID. NO: 6:

Arg Gly Trp Lys Leu Thr
5

SEQ ID. NO: 7:

Thr Thr Leu Val Leu Ser Gly Glu
5

SEQ ID. NO: 8:

Arg Phe Gly Ala Tyr Leu Val Ala His Lys Leu Met Ser Phe Glu Asp
1 5 10 15

SEQ ID. NO: 9:
Phe Ser Lys Ile Ile Gly Val Phe Thr Arg Ile Asp Asp Pro Glu Thr
1 5 10 15

Lys Arg Gln Asn Ser Asn Leu Phe Ser
20 25.

32. The method of Claim 31, wherein the amino acid sequence is:

Lys Val Leu Thr Ser Phe Thr Asn Lys Glu Leu Gln Ala Tyr Ala Lys
1 5 10 15

(SEQ ID. NO. 2).

33. The method of Claim 31, wherein the amino acid sequence is:

Ser Arg Asp Asp Asp Met Glu Thr Lys Arg Gln Asn Glu Asn
1 5 10
(SEQ ID. NO. 4).

34. The method of claim 30, further comprising an adjuvant.

35. The method of claim 34, wherein the adjuvant is selected from the group consisting of lipophilic muramyl dipeptide derivatives, nonionic block polymers, aluminum hydroxide, aluminum phosphate and Lipid A.

36. The composition of Claim 35, wherein the adjuvant is Lipid A.

37. The method of Claim 30, further comprising an "antigenic carrier" attached to the amino acid sequence so as to elicit an immune response against the amino acid sequence when administered to a human or animal.

38. The method of Claim 30, further comprising at least one lipophilic moiety attached to the amino acid sequence, and a liposome, wherein the lipophilic moiety is inserted into the liposome such that the amino acid sequence is exposed on the external surface of the liposome.

39. The method of Claim 38, wherein the lipophilic moiety has a long chain fatty acid with 10 to 24 carbon atoms in the lipid backbone.

40. The method of Claim 39, wherein the lipophilic moiety is selected from the group consisting of palmitic acid, stearic acid myristic acid, lauric acid, oleic acid, linoleic acid, and linolenic acid.

41. The method of Claim 40 wherein the lipophilic moiety is palmitic acid.

42. The method of claim 38, further comprising an adjuvant.

43. The method of claim 42, wherein the adjuvant is selected from the group consisting of lipophilic muramyl dipeptide derivatives, nonionic block polymers, aluminum hydroxide, aluminum phosphate and Lipid A.

44. The method of Claim 43, wherein the adjuvant is Lipid A.

45. A composition, comprising antibodies capable of specifically binding to one or more portions of P-glycoprotein that is externally exposed on the outer surface of a cell.

46. The composition of claim 45, wherein the antibodies specifically bind to an externally exposed portions of the P-glycoprotein selected from the group consisting of:

Gly Asn Met Thr Asp Ser Phe Thr Lys Ala Glu Ala Ser Ile Leu Pro
1 5 10 15

Ser Ile Thr Asn Gln Ser Gly Pro Asn Ser Thr Leu Ile Ile Ser Asn
20 25 30

Ser Ser Leu Glu Glu Glu
35

SEQ ID. NO: 1;

Lys Val Leu Thr Ser Phe Thr Asn Lys Glu Leu Gln Ala Tyr Ala Lys
1 5 10 15

SEQ ID. NO: 2;

Leu Ser Asn Glu Tyr Ser Ile Gly Glu
1 5

SEQ ID. NO: 3;

Ser Arg Asp Asp Asp Met Glu Thr Lys Arg Gln Asn Glu Asn
1 5 10

SEQ ID. NO: 4;

Gly Glu Met Thr Asp Ile Phe Ala Asn Ala Gly Asn Leu Glu Asp
1 5 10 15

Leu Met Ser Asn Ile Thr Asn Arg Ser Asp Ile Asn Asp Thr Gly Phe
20 25 30

Phe Met Asn Leu Glu Glu Asp Met Thr Arg Tyr Ala Tyr Tyr Tyr Ser
35 40 45

SEQ ID. NO: 5;

Arg Gly Trp Lys Leu Thr
5

SEQ ID. NO: 6;

Thr Thr Leu Val Leu Ser Gly Glu

SEQ ID. NO: 7;

Arg Phe Gly Ala Tyr Leu Val Ala His Lys Leu Met Ser Phe Glu Asp
1 5 10 15

SEQ ID. NO: 8; AND

Phe Ser Lys Ile Ile Gly Val Phe Thr Arg Ile Asp Asp Pro Glu Thr
1 5 10 15

Lys Arg Gln Asn Ser Asn Leu Phe Ser
20 25

SEQ ID. NO: 9.

47. The composition of Claim 46, wherein the antibody specifically binds the amino acid sequence:

Lys Val Leu Thr Ser Phe Thr Asn Lys Glu Leu Gln Ala Tyr Ala Lys
1 5 10 15

(SEQ ID. NO. 2)

48. The composition of Claim 46, wherein the antibody specifically binds the amino acid sequence:

Ser Arg Asp Asp Asp Met Glu Thr Lys Arg Gln Asn Glu Asn
1 5 10

(SEQ ID. NO. 4)

49. The composition of Claim 45, wherein the antibodies reduce multidrug resistance of cells having P-glycoprotein.

50. A method for reducing multidrug resistance in a human or animal comprising,

administering to a human or animal having multidrug resistance an amount of antibodies capable of specifically binding to one or more portions of P-glycoprotein

that are externally exposed on the outer surface of a cell effective for reducing the multidrug resistance.

51. The method of Claim 50 where the antibodies are directed against the amino acid sequence:

Lys Val Leu Thr Ser Phe Thr Asn Lys Glu Leu Gln Ala Tyr Ala Lys
1 5 10 15

(SEQ ID. NO. 2).

52. The method of Claim 50 where the antibodies are directed against the amino acid sequence:

Ser Arg Asp Asp Asp Met Glu Thr Lys Arg Gln Asn Glu Asn
1 5 10
(SEQ ID. NO. 4).

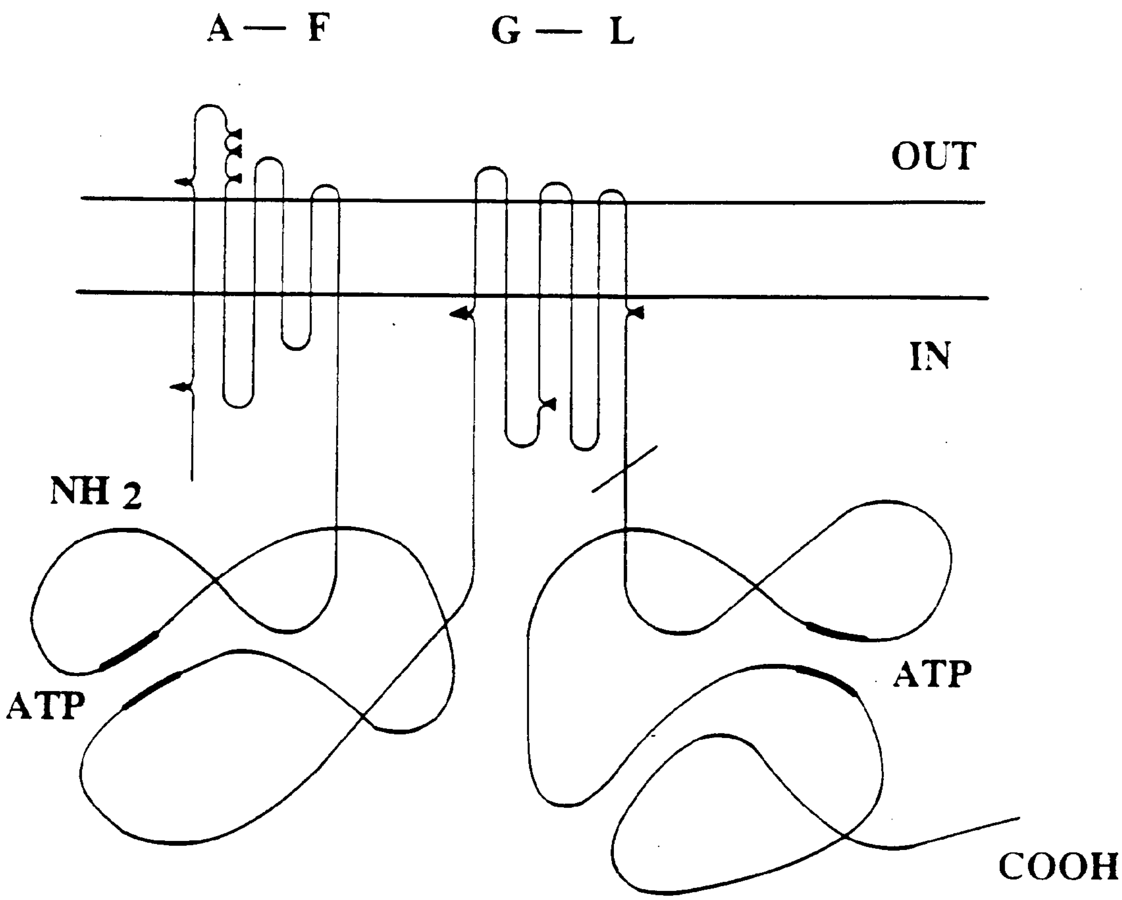


Fig_1

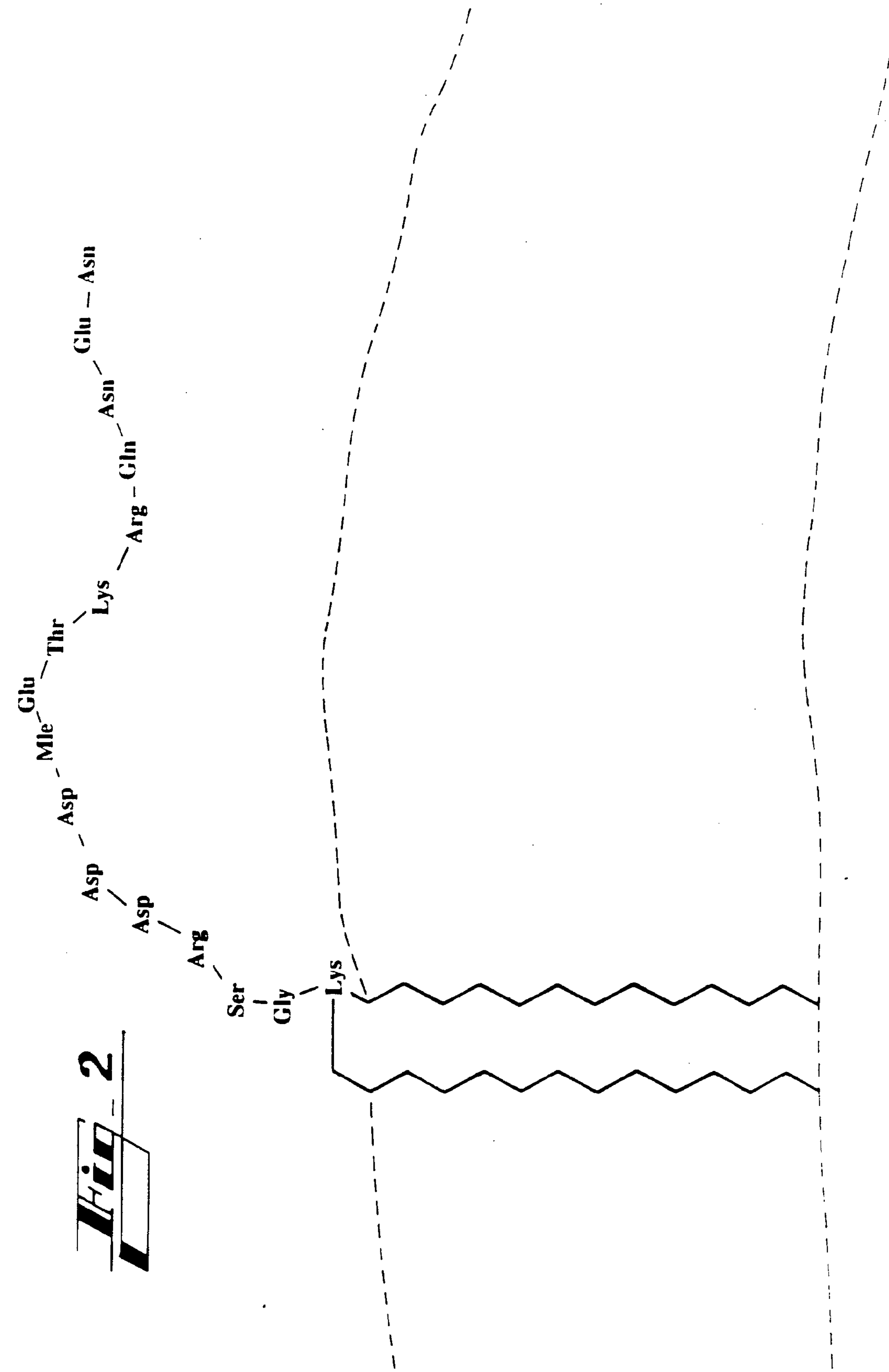


Fig. 2

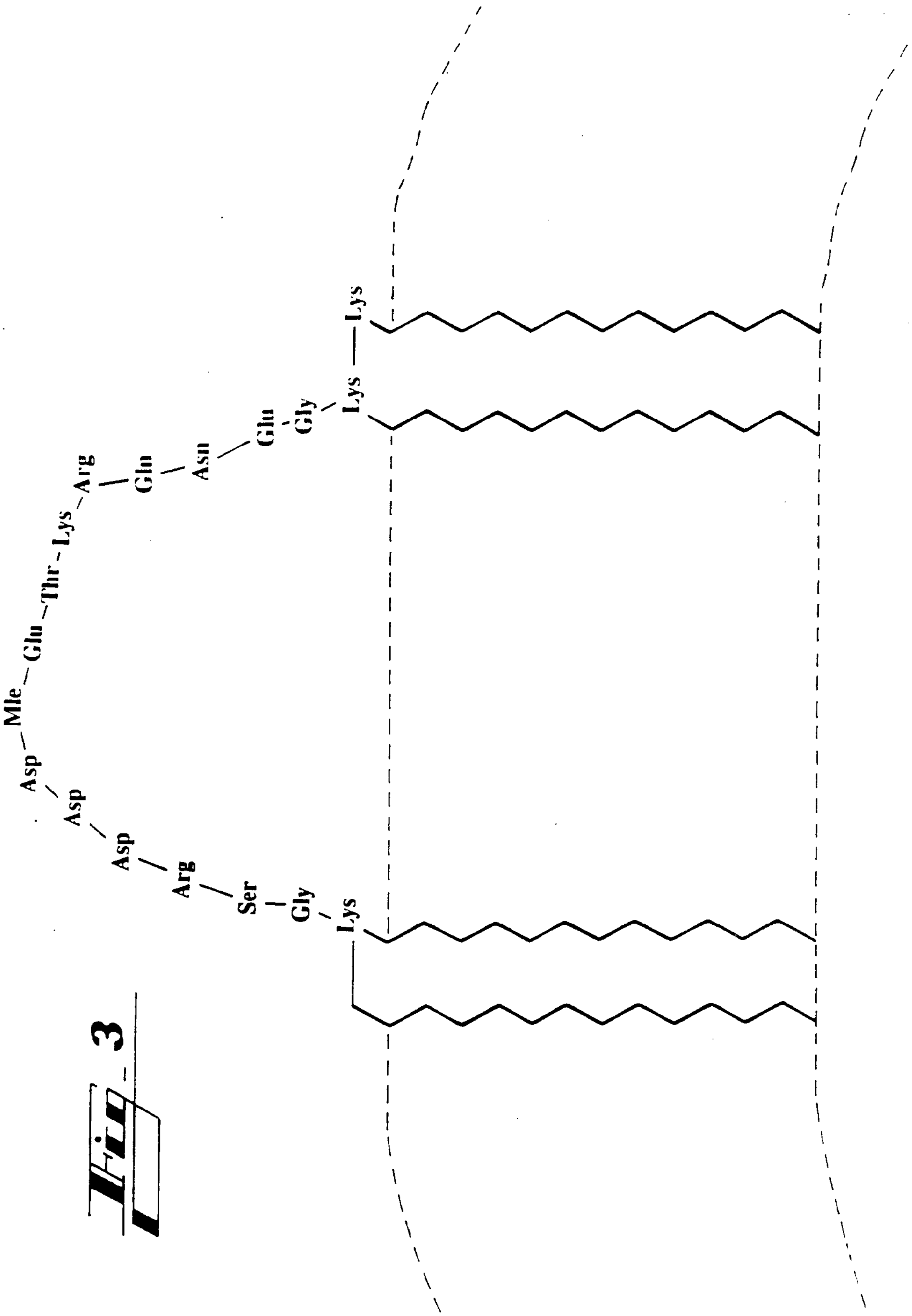
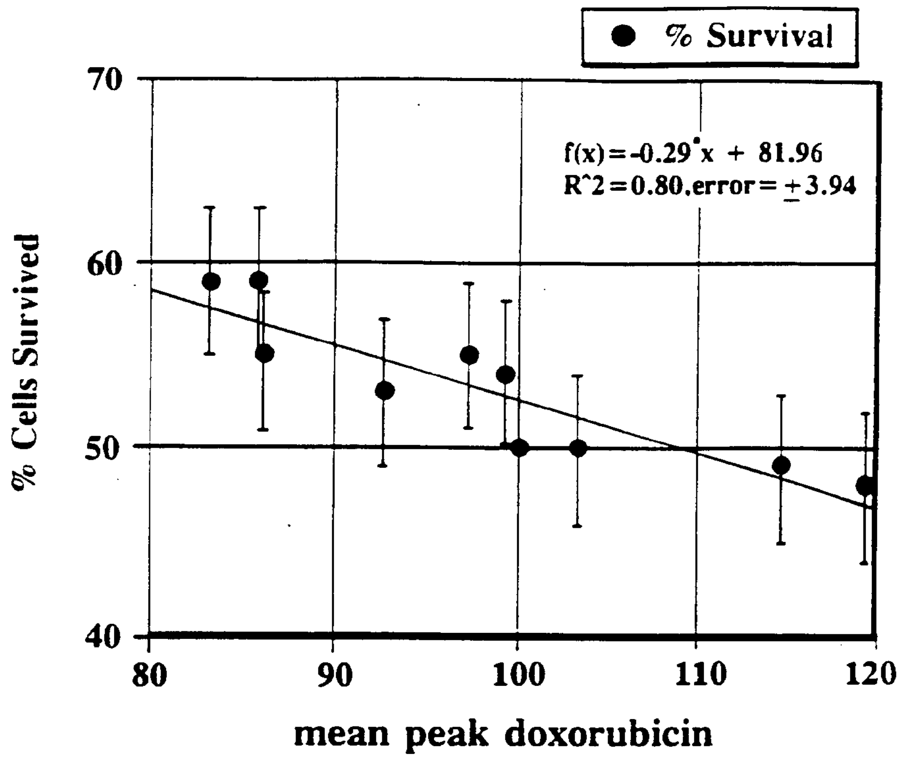
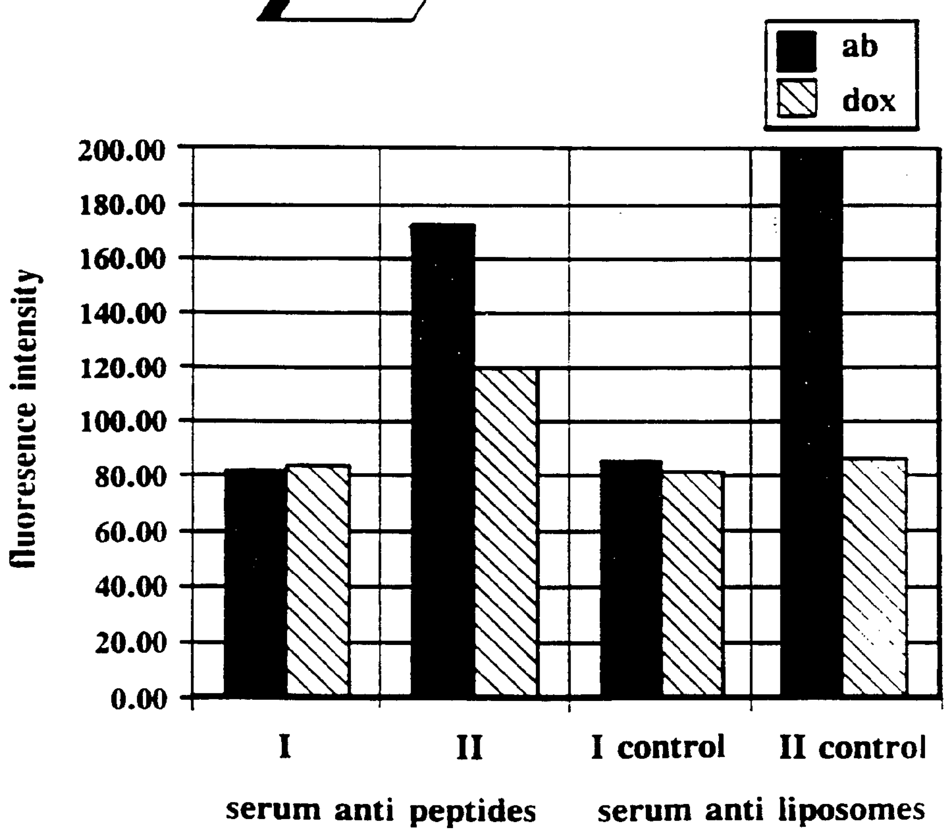

Lys
Lys
Gly
Glu
Asn
Gln
Arg
Lys
Thr
Glu
Mle
Asp
Asp
Asp
Arg
Ser
Gly
Lys

Fig. 3



% Survival
70
60
50
40
80
90
100
110
120
f(x)=-0.29*x + 81.96
R^2=0.80,error=±3.94
% Cells Survived
mean peak doxorubicin

Fig_4



ab
dox
200.00
180.00
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
fluoresence intensity
I
II
I control
II control
serum anti peptides
serum anti liposomes

Fig_5

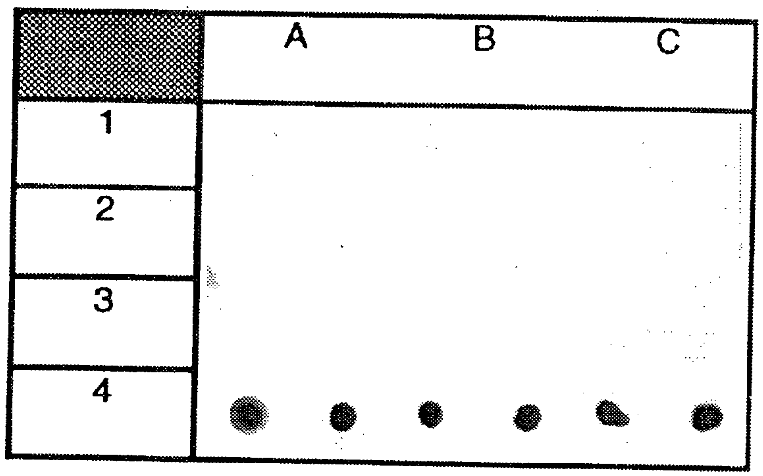


FIGURE 6A

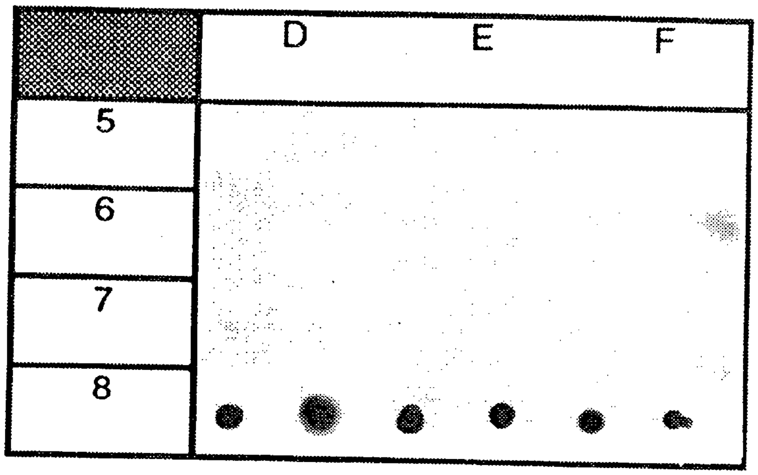


FIGURE 6B

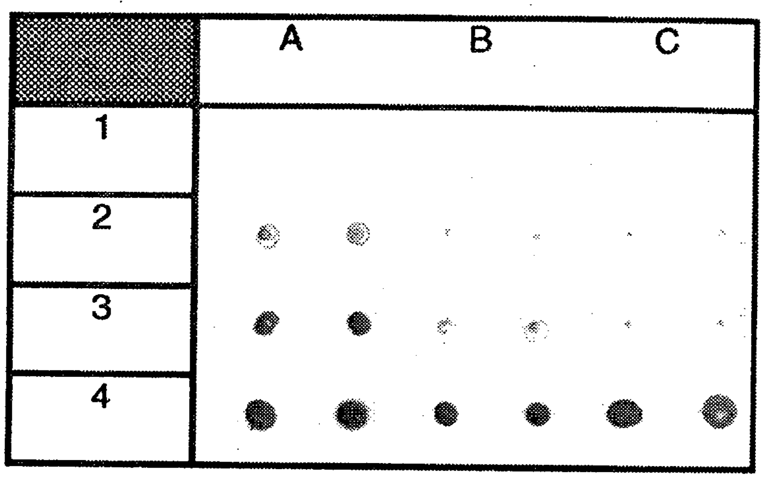


FIGURE 7A

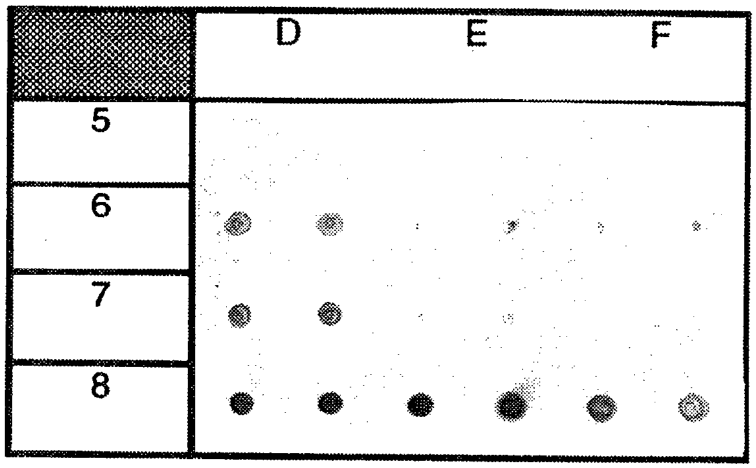


FIGURE 7B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 93/03073

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07K13/00 A61K39/00 A61K39/395 A61K9/127 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,87 05943 (BOARD OF TRUSTEES OF UNIVERSITY OF ILLINOIS) 8 October 1987 see page 40 - page 43 see figure 5 see claims 5,6,8	8,9,12, 30,31, 34,45, 46,49,50
A		15,37

X	BIOCHIMICA ET BIOPHYSICA ACTA vol. 1107, no. 1 , 11 June 1992 pages 105 - 110 SAEKI ET AL. 'Specific drug binding by purified lipid-reconstituted P-glycoprotein : dependence on the lipid composition' see the whole document	1,5,8, 12,15,45

	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search

18 March 1994

Date of mailing of the international search report

07 -04- 1994

Name and mailing address of the ISA

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Authorized officer

Gac, G

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 12173 (THE UNITED STATES OF AMERICA, THE SECRETARY, US DEPARTMENT OF COMMERCE) 23 July 1992 see page 9 - page 15 see page 21, line 26 - line 31 see page 24, line 4 - line 8 ---	8,12,15, 45
X	CELL vol. 47, no. 3 , 7 November 1986 pages 371 - 380 GROS ET AL. 'Mammalian multidrug resistance gene : complete cDNA sequence indicates strong homology to bacterial transport proteins' see the whole document	8-11,45
A		30-33, 49-52

X	CELL vol. 47, no. 3 , 7 November 1986 pages 381 - 389 CHEN ET AL. 'Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells' see whole document , especially page 384, Figure 7 page 387	8,9
A		30,31

P,X	WO,A,93 19094 (BOARD OF TRUSTEES OF THE UNIVERSITY OF ILLINOIS) 30 September 1993 see abstract see page 3 - page 7 see page 19 - page 20 see page 27, column 28 see examples 8-10 ---	45,49,50
X	PRINCESS TAKAMATSU SYMP. vol. 21 , 1990 pages 241 - 251 TSURUO T. 'Multidrug resistance : a transport system of antitumor agents and xenobiotics'	1,8,45, 49,50
Y		5-7, 12-14, 20-22, 27-29, 34-36, 42-44
	'xenobiotics and cancer' 1991 , ERNSTER ET AL. , LONDON, GB see page 243 see page 247 see page 248 ---	

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INTERNATIONAL SEARCH REPORT

Inte .onal Application No
PCT/EP 93/03073

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO,A,93 25700 (INSTITUTO SUPERIORE DI SANITA) 23 December 1993 see the whole document	8,12,30, 34,45, 49,50
Y	WO,A,90 01947 (THE LIPOSOME COMPANY INC.) 8 March 1990 see page 6 - page 10	5-7, 12-14, 20-22, 27-29, 34-36, 42-44
A	EP,A,0 267 781 (THE ONTARIO CANCER INSTITUTE) 18 May 1988 see the whole document	8-12,15, 30-34, 37,45-52
A	ANNALS OF THE NEW YORK ACADEMY OF SCIENCES vol. 660 , 28 October 1992 , NEW YORK pages 300 - 302 THIERRY ET AL. 'Liposomal delivery of antisense oligodeoxynucleotides : application to the inhibition of the multidrug resistance in cancer cells' see the whole document	1,8,23, 30
A	WO,A,92 00081 (CHIRON CORPORATION) 9 January 1992 see pages 9,11,12,14,20-30	1,5,6, 12,13, 15-18, 20,21, 27,28, 34,35, 37-40, 42,43
A	EP,A,0 356 339 (THE LIPOSOME COMPANY INC.) 28 February 1990 see the whole document	1,5,6, 23,27,28

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte .onal Application No
PCT/EP 93/03073

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-8705943	08-10-87	AU-B- 614489	05-09-91
		AU-A- 7286187	20-10-87
		EP-A- 0274482	20-07-88
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		JP-T- 4500203	16-01-92
		WO-A- 9001947	08-03-90
		WO-A- 9001948	08-03-90
		US-A- 5100662	31-03-92

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 93/03073

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claims 23-35, 37-44, 50-52 are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.